short ischemic pulses. In mitochondria, complex I produces ROS in the matrix, while, complex III mainly in the inter-membrane space. We hypothesized that the deleterious or protective action of ROS might depend by their site of production in the electron transfer chain (ETC).
We measured in heart isolated mitochondria ROS production (H_2O_2) with Amplex Red and the calcium-retention-capacity (CRC) with Calcium Green. CRC provides an indication of mitochondria viability as it measures the Ca^{2+} required to induce the opening of the mitochondrial permeability transition pore (mPTP). Specific substrates for complex I (glutamate/malate), complex II (succinate) that leads to ROS production in complex III, ETC inhibitors (rotenone and antimycin-A, pro-apoptotic agents), and MnSOD (catalyzes the conversion of superoxide into oxygen and H_2O_2) were used.
We found that CRC was much higher in mitochondria energized with succinate than with glutamate/malate. ROS production by glutamate/malate stimulation in mitochondria isolated 10 min after ischemia/reperfusion and mitochondria from non-ischemic heart is increased by ETC inhibitors. On the other hand, ROS production was diminished when in the same conditions were stimulated with succinate. MnSOD significantly increased ROS production when using glutamate/malate, while there was no significant effect with succinate.
These data strongly suggest that ROS produced in the matrix by complex I stimulation (glutamate/malate) is cardio-deleterious while when produced by stimulation of complex II (succinate) within the mitochondrial intermembrane space is cardioprotective. Supported by NIH and AHA.

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Visualization, Modeling, and Spatial Statistics of Mitochondrial Assembly in Adult Cardiomyocytes using Serial Block-Face Scanning Electron Microscopy
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Mitochondria are recognized as dynamic organelles, which constantly undergo morphological remodeling through fusion and fission processes. However, their geometric details including individual shape, size, and spatial distribution have not been quantitatively characterized in adult cardiomyocytes. Standard optical microscopy is unable to resolve neighboring mitochondria, which are intensely packed between myofilament bundles and narrow sub-sarcolemmal space. On the other hand, 3D reconstructs generated by serial thin-section transmission electron microscopy (EM) or EM tomography can only provide a limited field of view. We applied a novel advanced 3D electron microscopic technology, serial block-face scanning electron microscopy (SBFSEM), to adult mouse ventricular tissues, segmented mitochondria, and created geometric models of mitochondrial assembly. Substantially large volumes enclosing neighboring myocytes were imaged using SBFSEM. Subsequently, we applied spatial analysis tools using Spatstat R package to quantify organization of mitochondria. In cross-sectional slices generated from SBFSEM volumes, the boundaries of individual mitochondria were extracted and the centroid of each boundary was plotted. Analysis of the distribution of these centroids using a quadrat test of the intensity plot indicated borderline inhomogenity ($p=0.053$). The test was re-run after redrawing quadrats, now incorporating the location of the nucleus in the 3D stack. The results confirmed that mitochondria were clustered near the nucleus ($p=0.017$). The cell-wide inter-point interaction between mitochondrial centroids calculated by the L-function and the pair correlation function failed to support organized mitochondrial clustering. In summary, the study revealed the strength of the new integrated use of SBFSEM imaging and computational statistics to characterize and parameterize the spatial distribution of cellular organelles such as mitochondria that are dynamically remodeled under a physiological condition and more intensely in disease settings such as diabetic cardiomyopathy and heart failure.

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Bioenergetic Supply and Demand in the Cardiomyocyte
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The mechanisms that regulate the control of energy demand and supply in the myocardium are crucial for maintaining normal cardiac function. Although a number of mechanisms have been proffered by which mitochondrial supply of ATP can change to match varying workload in the myocardium, identifying the underlying regulatory pathways remains controversial. We describe an approach to studying this problem in which thermodynamically consistent mathematical models of the key energy-consuming processes in the cardiomyocyte (sarcoplasmic endoplasmic reticulum calcium ATPase (SERCA) [1], sodium pump [2] and the acto-myosin cross-bridge cycle [3]) are coupled to a model of mitochondrial ATP production within a whole-cell modelling framework for cardiac excitation-contraction coupling [4]. We use the model to investigate the metabolic stability hypothesis, wherein energy demand-supply homeostasis is maintained despite negligible variation in metabolite concentrations at varying cardiac workloads. We find that under physiological workloads cellular metabolite concentrations do not change significantly with increasing workload if a proposed feedback of inorganic phosphate onto mitochondrial oxidative phosphorylation is present, consistent with the proposition that Pi-regulation alone is sufficient to maintain metabolic homeostasis in the absence of other regulatory mechanisms. Finally, we use our model to address the empirically observed linearity of the cardiac ATP vs. Force-Length-Area curve (the cellular equivalent of the VO2 vs. Pressure-Volume-Area relationship). We show that the apparent linearity arises from the near irreversibility of the cross-bridge cycle, but that the linear relationship may disappear at cardiac workloads high enough that cellular metabolite concentrations start to vary.
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Characterization of Cardiomyocyte-Like Cells in Thoracic Blood Vessels
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Cardiomyocyte-like cells have been found in pulmonary veins (PV) of mammals, including humans. These cells are implicated as a possible origin of atrial fibrillation, yet their biological nature and physiological function remain poorly understood. We sought 1) to characterize the differentiation states of the cardiomyocyte-like cells in mouse PV during postnatal development, 2) to determine the distribution of cardiomyocyte-like cells in the vasculature, and 3) to investigate contractility of the venous cardiomyocyte-like cells in comparison with that of myocardium. Western blots using monoclonal antibodies recognizing cardiac muscle-specific proteins show that normal cardiac myofilament proteins are expressed at significant levels in PV and azygos vein. The expression of developmentally regulated isoforms of myosin and troponin is synchronized with that in heart. Transgenic mouse lines expressing β-galactosidase under the control of cardiac troponin T promoter show that the cardiomyocyte-like cells in PV reside singly and in clusters discontinuous from the atrial myocardium. Transmission electron microscopy found that the cardiomyocyte-like cells in PV have sarcomeric structures similar to that of ventricular cardiomyocytes. Isolated rat PV contracts upon electrical pacing and responds inotropically to isoproterenol, similarly to that of left atrium strips and ventricular papillary muscle. The paced contractile pattern of rat PV is distinct from the physiological contractions of vascular smooth muscle. Our data demonstrate that the cardiomyocyte-like cells in adult thoracic veins are at a highly differentiated state similar to that of cardiac myocytes in adult hearts. While the role of their excitability in the pathogenesis of atrial fibrillation remains controversial, the ectopic presence of differentiated cardiomyocyte-like cells provides a valuable model to understand the development and differentiation of cardiomyocytes.

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Analysis of Molecular Movement Reveals Lattice like Obstructions to Diffusion in Heart Muscle Cells
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Intracellular diffusion in muscle cells is known to be restricted. While characteristics and localization of these restrictions is yet to be elucidated, it has been established that ischemia-reperfusion injury reduces these restrictions. We extended raster image correlation spectroscopy and applied it to estimate directional anisotropy and coefficients of diffusion in rat cardiomyocytes. We determined that when comparing diffusion in cardiomyocytes to that in